Correlation of Leukorrhea and *Trichomonas vaginalis* Infection

Gweneth B. Lazenby, David E. Soper, Frederick S. Nolte

Department of Obstetrics and Gynecology and Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina, USA

*Trichomonas vaginalis* is a common sexually transmitted infection (STI) causing vaginitis. Microscopy has poor sensitivity but is used for diagnosis of trichomoniasis in resource-poor settings. We aimed to provide a more reliable diagnosis of trichomoniasis by investigating an association with leukorrhea. Women presenting for evaluation of vaginal discharge, STI exposure, or preoperative gynecologic examination were evaluated for *Trichomonas* infection. Vaginal pH was determined and microscopy was performed by the provider, who recorded the number of polymorphonuclear leukocytes (PMNLs) per epithelial cell and the presence of clue cells, yeast, and/or motile trichomonads. Leukorrhea was defined as greater than one PMNL per epithelial cell. Culture and a nucleic acid amplification test (NAAT) were used to detect *T. vaginalis*. Patients were evaluated for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using NAATs and bacterial vaginosis using Gram stains. Two hundred ninety-four women were enrolled, and 16% were found to have *Trichomonas* (46/294). *Trichomonas* infection was more common in parous non-Hispanic, black women, who reported low rates of contraceptive use (33% versus 17%; *P* = 0.02) and a STI history (85% versus 55%; *P* = 0.002). These women were more likely to report vaginal discharge (76% versus 59%; *P* = 0.02) and have an elevated vaginal pH (87% versus 48%; *P* < 0.001) and gonorrhea infection (15% versus 4%; *P* = 0.002). Leukorrhea was associated with a 4-fold-increased risk of *Trichomonas* infection. Leukorrhea on microscopy was associated with *Trichomonas* vaginitis. Patients with leukorrhea should be evaluated with more-sensitive tests for *T. vaginalis*, preferably NAATs, if microscopy is negative.

The prevalence of trichomoniasis in U.S. women is more than the combined prevalences of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections (1). Infection with *Trichomonas* is associated with a significant risk of morbidity in women, including pelvic inflammatory disease, adverse pregnancy outcomes (e.g., preterm labor and delivery), cervical dysplasia, infertility, increased risk of postoperative infection, and HIV acquisition and transmission (2–5). U.S. health care costs related to trichomoniasis approach 34 million dollars per year (1). *Trichomonas vaginalis* is a parasitic flagellate that adheres to and engulfs vaginal epithelial cells through phagocytosis. The resulting death of epithelial cells leads to inflammation within the genital tract and an increased number of polymorphonuclear leukocytes (PMNLs) in vaginal fluid (6). An increase in PMNLs noted on microscopy of vaginal secretions indicates an infectious or inflammatory process and may be a useful screening tool when evaluating vaginal discharge.

Leukorrhea refers to the presence of PMNLs in vaginal fluid, which can be quantified as the number of PMNLs seen on microscopy. Previous studies have defined leukorrhea based on the number of PMNLs per high-power field (hpf). Leukorrhea is considered present if there are more than one PMNL per oil immersion field (×1,000) or more than 10 PMNLs per hpf (×400) (7, 8). Leukorrhea has also been described according to the ratio of PMNLs to vaginal epithelial cells; more than 1 PMNL per epithelial cell is consistent with leukorrhea (9).

Noninfectious conditions, such as pregnancy, menstruation, or infertility, are associated with an increased number of PMNLs in vaginal fluid (10–12). Genital tract infections, such as *C. trachomatis* cervicitis and endometritis, are strongly associated with leukorrhea (8). This association of leukorrhea and *C. trachomatis* infection is true in pregnant and nonpregnant women. Leukorrhea is a strong predictor for *C. trachomatis* infection in nonpregnant and pregnant women (relative risk [RR], 59; 95% confidence interval [CI], 8 to 413; versus RR, 16; 95% CI, 7 to 32) (7). The absence of leukorrhea has a high negative predictive value (95%) for the diagnosis of upper genital tract infection, i.e., endometritis (8).

There are currently no studies in the English literature for which a correlation between leukorrhea in vaginal secretions and *Trichomonas* infection is reported. This information would be clinically useful when evaluating vaginal discharge and lower genital tract symptoms. Microscopy is the most common method used to diagnose trichomoniasis, but the sensitivity is poor (55%; 35 to 77%) (13–16). The presence of leukorrhea in the absence of motile trichomonads could potentially be presumed to be cervicitis and empirically treated without further evaluation for *Trichomonas*. A known significant relationship between leukorrhea on microscopy and *Trichomonas* might prompt clinicians to screen women in this clinical scenario for this common infection using more-sensitive methods.

The objective of this study was to determine an association between the presence of leukorrhea on microscopy of vaginal secretions and *Trichomonas* infection.

**MATERIALS AND METHODS**

The study was approved by the Medical University of South Carolina Institutional Review Board (HR no. 19180). Two hundred ninety-four
women presenting to our urban academic practice for a gynecologic exam were enrolled in the study. Patients were eligible for the study if they had undergone an Aptima Combo 2 assay for *N. gonorrhoeae* and *C. trachomatis* (Gen-Probe Inc., San Diego, CA) as part of a routine exam or an evaluation for vaginal discharge or sexually transmitted infection (STI) exposure. Women were excluded if they were pregnant, HIV positive, and/or had a gynecologic malignancy, vaginal bleeding, or an active genital herpes infection. Women were also excluded if they had undergone surgery or were treated for pelvic inflammatory disease, appendicitis, vaginitis, or a STI within 28 days of presentation. The following patient information was collected: age, race, marital status, parity, first day of last menstrual period, contraceptive use, condom use, douching practices, partner gender preference, number of partners in the last year, history of STIs, and history of abnormal cervical cytology.

During a gynecologic examination, the following tests were performed: vaginal pH, Gram stain, *T. vaginalis* culture (InPouch, Biomed Diagnostics, White City, OR), nucleic acid amplification tests (NAATs) for *N. gonorrhoeae, C. trachomatis*, and *T. vaginalis* (Aptima Combo 2 and TV analyte specific reagent; Gen-Probe Inc., San Diego, CA), and microscopy of vaginal secretions. The microscopy specimen was prepared by placing a vaginal swab into a test tube containing a small amount of normal saline (approximately 3 drops). A drop of this solution was then placed on a glass slide and reviewed under an hpf (×400). When reviewing microscopy of vaginal secretions, examiners noted the presence of PMNLs, clue cells, yeast, and/or motile trichomonads. Leukorrhea on microscopy was defined as >1 PMNL per epithelial cell per hpf (×400). Clinicians performing microscopy were Clinical Laboratory Improvement Amendments (CLIA) certified and participated in competency assessment and proficiency testing with guidance from the clinical laboratories’ point-of-care testing team.

Gram staining of vaginal secretions, *T. vaginalis* cultures, and NAATs were performed by the Medical University of South Carolina Microbiology and Molecular Pathology labs. Gram stains were interpreted using Nugent’s criteria to diagnose bacterial vaginosis (BV) (17). The number of PMNLs per hpf on Gram staining was rated using the following scale: 0 PMNLs/hpf, 1 to 5 PMNLs/hpf, and more than 5 PMNLs/hpf. *T. vaginalis* cultures were incubated at 37°C and inspected every other day up to 5 days for the presence of motile trichomonads.

Vaginal swab samples were transported to the laboratory for NAATs using the Aptima transport medium. The Aptima Combo 2 assays for *N. gonorrhoeae* and *C. trachomatis* were performed and interpreted according to the manufacturer’s recommendations using the semiautomated direct tube sampling (DTS) system. The Aptima *T. vaginalis* analytic-specific reagent was used to develop a NAAT for *T. vaginalis* on the DTS system (4). Aliquots of the Aptima vaginal swab transport medium were stored for up to 12 months at −70°C before testing for *T. vaginalis*. Specimens with relative-light-unit values of <100,000 were considered negative, and those with ≥100,000 relative-light-unit values were considered positive.

Statistical analyses were performed using the SAS 9.3 software program (SAS, Cary, NC). Continuous variables (age and parity) were analyzed using Student’s *t* test to compare means and the Wilcoxon rank sum test to compare medians. For dichotomous variables, chi-square and Fisher’s exact tests were used to compare women with and without *Trichomonas* infection. Univariate logistic regression analyses were used to determine factors associated with *Trichomonas* infection. Variables with a *P* value of ≤0.2 were included in multivariate logistic regression analyses. Kappa statistics were calculated to determine the agreement between *Trichomonas* culture and NAATs. Chi-square tests were used to calculate the sensitivity, specificity, and positive and negative predictive values of culture, visualization of motile trichomonads, and leukorrhea on microscopy for the diagnosis of trichomoniasis.

### RESULTS

Two hundred ninety-four women, ages 16 to 67, were enrolled in this study. More than 60 percent of the subjects presented for evaluation of a vaginal discharge (179). The majority of subjects were non-Hispanic, black (216) and currently unmarried (260). Ninety-eight percent of women reported being exclusively heterosexual (287), and more than half had a history of an STI (175). Most women reported using a method of contraception, including condoms (234). Other patient characteristics are listed in Table 1.

*Trichomonas* testing results were available for almost all 294 women enrolled in the study: 292 *T. vaginalis* cultures and 247 nucleic acid amplification tests. Sixteen percent (46) of the study subjects had a positive *Trichomonas* test result. Women with a *Trichomonas* infection were more likely to be parous (88% versus 64%; *P* = 0.002) and non-Hispanic black (93% versus 70%; *P* = 0.0004). They were less likely to use any form of contraception (33% versus 17%; *P* = 0.02), but rates of condom use were similar in the two groups (4% versus 8%; *P* = 0.5). Women with *Trichomonas* infection were more likely to report a history of STIs (85% versus 55%; *P* = 0.002) (Table 2).

According to Nugent’s criteria, 41% of women in the study had BV (119/289). Five Gram stains could not be scored due to inadequate specimen collection. The rates of BV determined by Nugent’s criteria and high-power microscopy findings of clue cells were equal (both 41%), but the agreement between these tests was low (kappa statistic = 0.5). The rates of BV were similar among the women infected with *Trichomonas* versus those not infected (43% versus 41%; *P* = 0.7). PMNLs were noted on 71% of all Gram stain specimens. Compared to uninfected women, women with *T. vaginalis* were more likely to have PMNLs on Gram stain-

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) (mean ± SD)</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Parity, median (interquartile range)</td>
<td>1.4 (0–8)</td>
</tr>
<tr>
<td>% of patients (no. of patients/total) with characteristic</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic, black race</td>
<td>74 (216/293)</td>
</tr>
<tr>
<td>Hispanic race</td>
<td>0.6 (2/293)</td>
</tr>
<tr>
<td>Single marital status</td>
<td>90 (260/290)</td>
</tr>
<tr>
<td>Exclusively heterosexual partner preference</td>
<td>98 (287/292)</td>
</tr>
<tr>
<td>Vaginal douching</td>
<td>16 (46/286)</td>
</tr>
<tr>
<td>Regular menstrual cycles</td>
<td>63 (170/272)</td>
</tr>
<tr>
<td>Currently not using contraception (including condoms)</td>
<td>20 (58/292)</td>
</tr>
<tr>
<td>Report condoms only for contraception</td>
<td>7 (22/294)</td>
</tr>
<tr>
<td>Hormonal contraception</td>
<td>58 (169/292)</td>
</tr>
<tr>
<td>Surgical contraception</td>
<td>14 (41/292)</td>
</tr>
<tr>
<td>History of abnormal cervical cytology</td>
<td>17 (47/275)</td>
</tr>
<tr>
<td>Multiple sexual partners</td>
<td>24 (68/278)</td>
</tr>
<tr>
<td>Patient reported vaginal discharge</td>
<td>61 (179/292)</td>
</tr>
<tr>
<td>History of one prior sexually transmitted infection (STI)</td>
<td>60 (175/292)</td>
</tr>
<tr>
<td>History of more than one STI</td>
<td>49 (84/170)</td>
</tr>
<tr>
<td>History of <em>Trichomonas</em> infection</td>
<td>40 (66/170)</td>
</tr>
<tr>
<td>History of <em>Chlamydia</em> infection</td>
<td>62 (106/170)</td>
</tr>
<tr>
<td>History of gonorrhea infection</td>
<td>37 (61/166)</td>
</tr>
<tr>
<td>History of genital herpes</td>
<td>12 (21/170)</td>
</tr>
</tbody>
</table>

- Some patient characteristic data were not available for all participants.
- Regular menstrual cycles defined as monthly menses every 21 to 35 days.
- Surgical contraception may include female tubal ligation or male vasectomy.
- Defined as >1 partner in the 12 months preceding evaluation.
The visualization of any PMNLs on Gram staining (hpf, ×1,000) was a more-sensitive test for *Trichomonas* infection than leukorrhea on microscopy (hpf, ×400) (90% versus 78%). However, the specificity of Gram staining was lower than that of leukorrhea on microscopy. The PPV for leukorrhea assessed by Gram staining versus the PPV for assessment by microscopy were similar, 21 and 25%, respectively (Table 4).

**DISCUSSION**

The incidence of *Trichomonas vaginalis* infection among the women in this study population (16%) is five times higher than...
TABLE 4 Diagnostic techniques for the detection of *Trichomonas* compared to nucleic acid amplification tests

<table>
<thead>
<tr>
<th>Method or target</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>% PPV</th>
<th>% NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>93 (38/41)</td>
<td>100 (205/205)</td>
<td>100 (38/38)</td>
<td>99 (205/208)</td>
</tr>
<tr>
<td>Motile trichomonads</td>
<td>51 (21/41)</td>
<td>98 (200/204)</td>
<td>84 (21/25)</td>
<td>91 (200/220)</td>
</tr>
<tr>
<td>Leukorrhea on microscopy</td>
<td>78 (32/41)</td>
<td>51 (102/199)</td>
<td>25 (32/129)</td>
<td>92 (102/111)</td>
</tr>
<tr>
<td>Motile trichomonads or leukorrhea</td>
<td>78 (32/41)</td>
<td>53 (109/207)</td>
<td>25 (32/130)</td>
<td>92 (109/118)</td>
</tr>
<tr>
<td>PMNLs on Gram staining</td>
<td>90 (37/41)</td>
<td>33 (68/207)</td>
<td>21 (37/176)</td>
<td>94 (68/72)</td>
</tr>
</tbody>
</table>

*Parenthetical numbers are no. of correct results/no. tested. PPV, positive predictive value; NPV, negative predictive value.

InPouch *Trichomonas* culture was more sensitive and specific than microscopy for the diagnosis of *Trichomonas*.

Leukorrhea, ≥1 PMNL per epithelial cell per hpf (×400).

PMNLs on Gram staining, ≥1 PMNL per hpf (×1,000).

The reported prevalence of *Trichomonas* infection in American women (1). In our population, *Trichomonas* vaginitis was associated with leukorrhea on microscopy (>1 PMNL per epithelial cell per hpf, ×400) and PMNLs on Gram staining (>1 PMNL per hpf, ×1,000). Both of these findings have lower sensitivity for *Trichomonas* infection than for culture (93% versus 78% and 90%, respectively) but improved sensitivity over that for motile trichomonads on microscopy (51%). Negative predictive values for motile trichomonads, leukorrhea, PMNLs on Gram staining, and culture were comparable. In comparison to NAATs for the diagnosis of trichomoniases, neither leukorrhea on microscopy nor Gram staining was specific, and both had low positive predictive values (Table 4).

The incidence of *Trichomonas* infection in this group of women is likely not reflective of the general population. Our obstetrics-gynecology (OBGYN) residency women’s health practice provides care to mostly young, minority women with a high rate of STIs. The mean age of these study subjects was 28 (±9) years, the majority of women (74%) were non-Hispanic black, and more than half (60%) reported a history of at least one STI (Table 1).

In a national survey of American women, low level of education (high school or less), poverty, increasing number of sexual partners, early age of first sexual intercourse, and douching were associated with *Trichomonas* infection (1). In our population, douching and reporting more than one sexual partner in the past 12 months was not associated with trichomoniases. These findings are likely due to the high proportion of patients reporting douching and multiple partners in the overall study group. Risk factors associated with *Trichomonas* infection among the women in this study were parity, non-Hispanic black race, lack of contraceptive use, including condoms, and a history of a STI. These factors associated with increased risk of STIs are consistent with those in previous reports with the exception of an increased number of sexual partners (Table 2) (18).

Exam findings associated with *Trichomonas* vaginitis in our population were an elevated vaginal pH (>4.5), leukorrhea on microscopy, PMNLs on Gram staining, and gonorrhea infection. Patient report of vaginal discharge was associated with trichomoniases in univariate analysis, but this association was attenuated in multivariate analysis when accounting for other exam findings: pH, PMNLs on Gram staining, and cervicitis (Table 3). The poor association between symptomatic vaginal discharge is consistent with other population-based studies of *Trichomonas* infection (1). The high rate of asymptomatic infections in women supports the notion that clinicians should not limit evaluations for *Trichomonas* vaginitis to microscopy in those reporting vaginal discharge (19). We suggest that clinicians consider the factors associated with *Trichomonas* infection in order to identify patients at risk and use tests more sensitive than microscopy, such as culture or NAATs, to diagnose *Trichomonas* infection.

Similar to studies of other genital tract infections, leukorrhea in vaginal secretions was associated with *Trichomonas* infection. This is physiologically plausible, because *Trichomonas* causes increased inflammation within the vaginal environment and has been found to “attract” PMNLs (6). Specifically, CD4 lymphocytes are recruited to the site of *Trichomonas* infection (20).

In the absence of leukorrhea on microscopy (>1 PMNL per epithelial cell), *Trichomonas* infection was unlikely (OR, 0.3; 95% CI, 0.1 to 0.8). Although gonorrhea cervicitis was associated with an increased risk of *Trichomonas* infection, this did not impact the association between leukorrhea and *Trichomonas* vaginitis. When controlling for the presence of gonorrhea, *C. trachomatis* infection, and bacterial vaginosis, patients were 4 times more likely to have *Trichomonas* if leukorrhea was noted on microscopy (OR, 3.6; 95% CI, 1.6 to 7.6). Our findings suggest that the presence of leukorrhea on microscopy should increase clinicians’ suspicion of *Trichomonas* infection and lead to further evaluation with more-sensitive tests if microscopy is negative for motile trichomonads.

The Aptima *T. vaginalis* analyte-specific reagent used in this study has emerged as the most sensitive test for detection of *Trichomonas* infections (4, 21–23). Because concomitant *C. trachomatis* and *N. gonorrhoeae* infections are common in patients with trichomoniases, a panel of NAATs for these three infectious agents would be ideal for comprehensive STI diagnosis. The Aptima *T. vaginalis* assay was recently cleared by the FDA for diagnostic use. This should greatly increase availability of the test, with cost and turnaround time for results similar to those of the Aptima *C. trachomatis* and *N. gonorrhoeae* assay.

The association between leukorrhea and *Trichomonas* vaginitis in our population is both physiologically plausible and likely applicable to other populations seeking evaluation for vaginal discharge or gynecologic exam. Our study subjects are not representative of a general population given the young age of participants, high rates of reported and concurrent STIs, and disproportionate representation of minorities. However, this population is similar to others studied in urban centers, STI clinics, and family planning centers. It is in these low-resource settings that microscopy is often used for the diagnosis of *Trichomonas*. Our findings suggest that the presence of leukorrhea on microscopy should increase clinical suspicion for *Trichomonas* vaginitis, prompting additional tests, and may enhance identification of this common and morbid infection.
ACKNOWLEDGMENTS

We acknowledge Gen-Probe, Inc., for their support and funding of this research.

We thank the staff of the MUSC Microbiology and Molecular Pathology labs for their tireless work in analyzing the additional specimens required for this study. We especially recognize April Kegl.

The coinvestigator Fredrick Nolte has been a member of the Gen-Probe Scientific Advisory Board from 2007 to the present. The annual compensation associated with this activity is approximately $10,000. Gen-Probe Scientific Advisory Board from 2007 to the present. The annual compensation associated with this activity is approximately $10,000.

We especially recognize April Kegl.

The coinvestigator Fredrick Nolte has been a member of the Gen-Probe Scientific Advisory Board from 2007 to the present. The annual compensation associated with this activity is approximately $10,000. Gen-Probe Scientific Advisory Board from 2007 to the present. The annual compensation associated with this activity is approximately $10,000.

We thank the staff of the MUSC Microbiology and Molecular Pathology labs for their tireless work in analyzing the additional specimens required for this study. We especially recognize April Kegl.

We acknowledge Gen-Probe, Inc., for their support and funding of this research.

REFERENCES